Low success of controlled pollination in *Eucalytpus marginata* (jarrah)

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Abstract

The success of controlled pollination techniques was investigated for clones and wild trees of *Eucalyptus marginata*. The number of pollen tubes in the style was used as a measure of pollination success. The stigma was receptive from 3–11 days after anthesis, but it appeared that stigmatic receptivity may be highest from 4–7 days after anthesis. Flowers were isolated using aluminium foil squares, and fresh pollen was found to be more effective for pollination success than processed pollen. Results were inconclusive comparing self- to cross-pollination success, and no difference was found comparing pollination success in clones and wild trees. However clones generally tended to flower earlier than the wild trees, whatever their origins, and very few mature fruits resulted from any controlled pollinations in jarrah clones. Clones also produced a lower proportion of mature capsules from the flowers produced (Wheeler 2004)

Keywords: pollination, *Eucalyptus marginata*, stigma, style

Introduction

Control-pollination techniques have been developed in several species of *Eucalyptus* as a tool to assist in selective breeding. Controlled pollination has been used to improve seed yield (Harbard *et al.* 1999; Moncur 1995), control the level of outcrossing in seed orchards and improve breeding through knowledge of fertility and compatibility, achieve interspecific hybridisation, and study self-incompatibility levels in *Eucalyptus* species. Detailed knowledge of the pollination biology of *Eucalyptus marginata* (jarrah) is essential to design an effective seed orchard, or to conduct controlled pollination for genetic improvement of trees. This study was undertaken to assist in the development of control-pollination techniques in *E. marginata*, which could be used for the breeding of improved lines.

The floral structure of jarrah is similar to other *Eucalyptus* subgen. *Eucalyptus* species, such as *Eucalyptus stellulata* (House 1997, Fig. 1a), and *E. regnans* (Griffin 1980; Griffin & Hand 1979; Griffin *et al.* 1987). Flowers per umbel range between one and eleven, and trees do not usually flower prolifically every year, but have one good flowering season every two or three years. Flower buds are always seen in the autumn but can drop off later in the year.

Anthesis occurs in most eucalypt species 3–28 days prior to the stigma of the same flower being receptive (Griffin & Hand, 1979; Oddie & McComb 1998; Williams 1999). The style elongates following anthesis (operculum lift). While individual flowers are protandrous this does not prevent pollen from one flower pollinating and fertilising another flower on the same tree, and there is evidence that this occurs in most of the species studied (Elridge *et al.* 1993; Moncur & Boland 1989; Potts and Cauvin 1988; Pryor 1951, 1976).

Detection of stigma receptivity is essential for successful controlled pollination and different indicators of stigma receptivity have been described. The style in *Eucalyptus regnans* lengthens when approaching the receptive period, and the surface of the stigma expands (Griffin & Hand 1979). Receptivity in this species was determined (by the lengthening of the stigma and increased exudate on the stigma) to be between 10 and 14 days after anthesis. Stigma receptivity in three species in Section *Bisectaria* was marked by stigma secretions, coinciding with the greatest number of pollen grains adhering to the stigma surface (Ellis & Sedgley 1992). In *E. woodwardii* (*Eucalyptus* subgen. *Symphomyrtus*), no style elongation was observed during the receptive period, but stigma secretions were maximised when it was most receptive, seven days after anthesis (Sedgley & Smith 1989).

The method most often used for controlled pollination in eucalypts involves emasculation and isolation of flower buds, and frequently, pollen storage (Moncur, 1995). Low fertilisation rates have occurred in several species, and sometimes two controlled pollinations produce more seed. Griffin *et al.* (1987) reported many more fertilised ovules in *E. regnans* after successive pollinations two days apart. Pollen collection is usually from buds at operculum lift stage, and the pollen is sieved and placed in a vial, and stored at or below 0°C. Griffin *et al.* (1982) reported storing pollen from *E. regnans* satisfactorily at room temperature for 36 days, after grinding the anthers to release the pollen in a glass tissue grinder with distilled water. The homogenate was then filtered through a nylon cloth (37μm pore size) then

a millipore filter (3µm) and the pollen on the filter dried over silica gel. Turner *et al.* (1994) kept branches with buds in water for three to five days in the laboratory so that the buds could open and pollen easily collected for processing.

In *E. marginata* pollination is affected by a range of insect and bird vectors (Yates *et al.* 2005). Purple crowned lorikeets have been observed feeding on *E. marginata* flowers (House 1997). A range of insect species have been seen to visit *E. marginata* flowers, such as flies, bees, wasps, beetles and moths (Yates *et al.* 2005), in common with other *Monocalypts* such as *E. regnans* (Griffin & Ohmart 1986). Wind pollination is regarded as being unlikely as a major pollinating vector in *Eucalyptus*. Pollen is thought to be dispersed up to approximately 100 metres (Eldridge *et al.* 1993), and in *E. kochii* sometimes up to 500 metres (Byrne, *pers. comm.*), and, given the evidence from certain natural hybrids, apparently much further in some species (Potts *et al.* 2003).

While it could be expected that similar pollination rates could be seen in *E. marginata* as other eucalypts, as well as capsule production, it is not necessarily the case. This study, while following similar techniques to successful methods used in other species, is surprising in the low levels of pollination success achieved.

Materials and Methods

Trees used

Wild trees (endemic *E. marginata* trees) in the Harry Waring Mammal Reserve (32° 9' 47"S, 115° 49' 22"E), at Wattleup, Western Australia (WA) and clones (developed using tissue culture techniques for resistance to *Phythophthora cinnamomi*, McComb *et al.* 1996) growing at Murdoch University, WA (32° 2' 54"S, 115° 50' 46"E) and Marrinup Orchard, (32° 42' 5"S, 116° 3' 5"E) near Dwellingup, W.A. were used for control pollination experiments. Wild trees were chosen for their similar size to the clonal trees.

Stigmatic Receptivity and Pollination Success

Stigmatic receptivity in *Eucalyptus marginata* was first investigated using clones growing at Murdoch University during November 1998 and February 1999 (hereafter referred to as 1999). This work was further developed during September, October, and November, 2000, and in September/October, 2001 (referred to hereafter as 2000, 2001). Trials were undertaken to detect when stigmas were receptive. Stigma receptivity was determined by assessing the number (and sometimes the length) of pollen tubes in styles observed under microscopic UV light, as outlined in Sedgley & Smith (1989), and Potts and Gore (2000). Numbers of pollen tubes were estimated within a range of five (0 – 5, 5 – 10, etc.) because of the difficulty in making exact counts. Styles were collected 24–72 hours following pollination, fixed in Farmer's Fluid (Acetic Acid: Ethanol 1:3) for approximately one hour, then stored in 70% ethanol. After rinsing in distilled water the styles were slit along their length, then placed in 5% sodium sulphite and autoclaved. When cool, the styles were again rinsed in distilled water, the epidermis was removed, the style with the stigma placed on a microscopic slide in 1% aniline blue, and gently squashed under a cover slip. This tissue was observed under a fluorescence microscope for pollen tubes (Fig. 1d).

Time of stigmatic receptivity was tested in emasculated and intact flowers by pollinating 0 – 11 days after anthesis in 1999 and 2 – 7 days after anthesis in 2000, 2001 (Table 1). Pollination success variation within the flowering season was observed by comparing pollination success at the early, middle and late periods of the flowering season (20 flowers for two clones for each of the three times using processed pollen). A trial was also conducted to determine whether multiple pollinations of the same flower were advantageous. In jarrah, the flowers were pollinated on day four followed by a repeat pollination on day five after anthesis (20 flowers for two clones using fresh pollen).

Tests of pollination techniques

Preliminary work was conducted in 1999 to test stigmatic receptivity and emasculation and isolation techniques. In 2000 and 2001 a number of trials were conducted to test various aspects of the pollination process using two clones, and two wild trees.

Two pollination techniques were compared. These are: the 'processed' technique after Moncur (1995), and the 'fresh' technique. In each case pollen from several genotypes was used for cross-pollination. The 'processed' technique involved brushing the flowers of several genotypes over a 200mm sieve and collecting pollen in a small bottle with a rubber stopper and storing it at 4°C or at room temperature. Pollen was applied to the stigma using the rubber stopper or a toothpick. Secondly the 'fresh' technique involved wiping a freshly picked flower with the operculum removed across a receptive style. Each style was wiped with several buds from several genotypes. Branches with buds were sometimes picked and kept in water for up to five days in the laboratory, and the flowers used when they were at operculum lift stage. Only pollen with viability over 50% was used, determined by using the method described in Wheeler and McComb (2006). This technique involves germinating fresh pollen *in vitro* using the wettable cellophane technique (Alexander and Ganeshan 1989) with a germination medium of 300 ppm hydrated calcium nitrate, 200 ppm hydrated magnesium sulphate, 100 ppm potassium nitrate and 150 ppm boric acid in 25% maltose (after Brewbaker & Kwack 1963), or a medium of 25% maltose with 150 ppm boric acid (McComb unpub.). Cultures were incubated for 24 – 48 hours, then the pollen tubes were counted under a light microscope.

The clones were tested for differences between self- and cross-pollinated flowers in the numbers of pollen tubes, in the season of 2000, and in 2001 selfed and cross pollinations compared wild trees to clones. To test whether damage to the flowers from the removal of the anthers reduces pollination success, a comparison of pollination success using emasculated and non-emasculated flowers was conducted.

Isolation methods

In 1999 and 2000, the bagging method for isolation was based on Moncur (1995), using double crispy-wrap

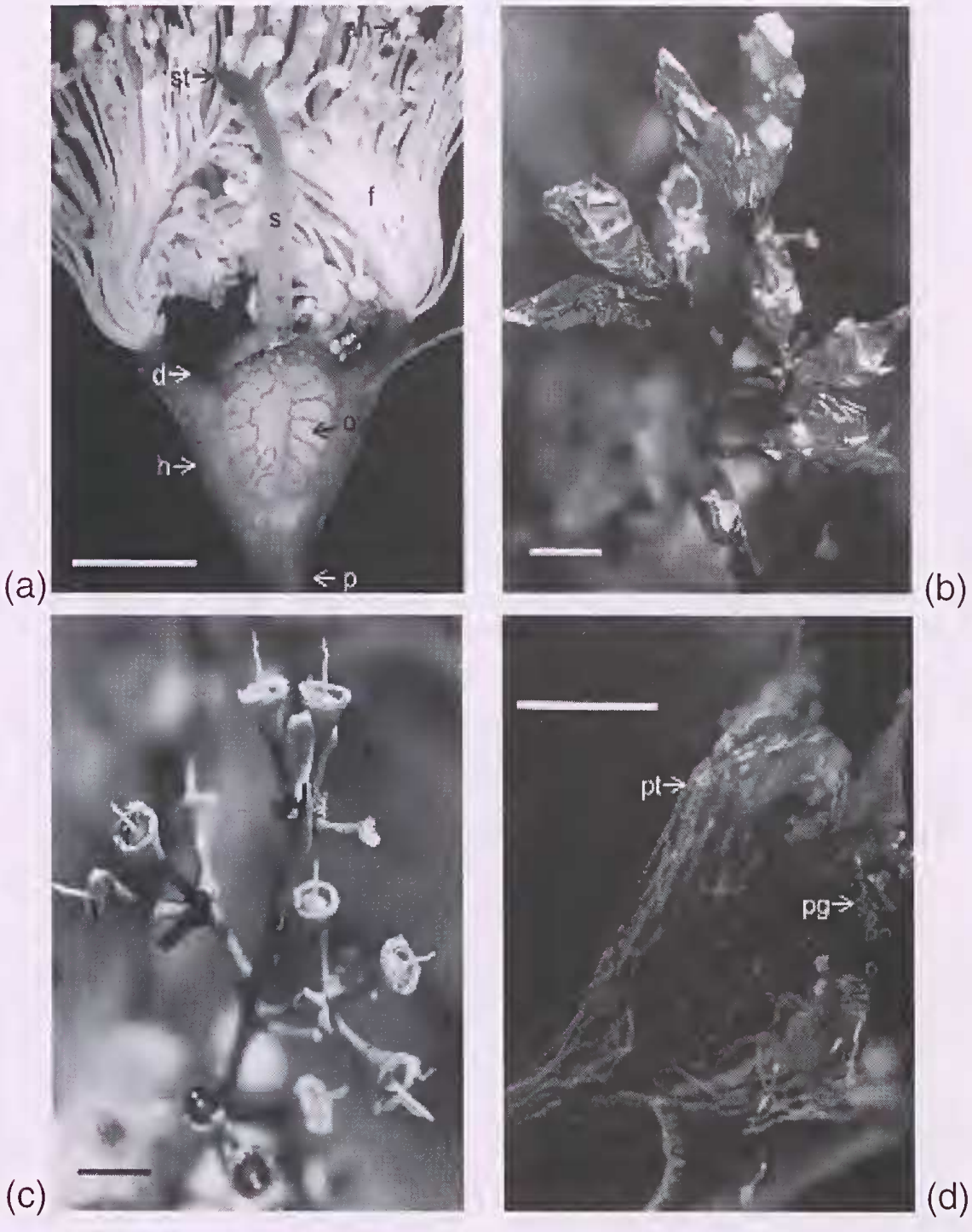

Figure 1. (a). Longitudinal mid-section of a *Eucalyptus marginata* flower at anthesis. Ovule walls have been digitally enhanced. St = stigma, s = style, f = filament, an = anther, d = disc, h = hypanthium, ov = ovule, p = pedicel. Bar = 5 mm. (b). Emasculated flowers of *E. marginata* that have been isolated with foil squares. Bar = 15 mm. (c). Emasculated flowers of *E. marginata*. Extra flowers have been pruned from the branch for the photograph. Bar = 10 mm. (d). Fluorescence image of a squashed *Eucalyptus marginata* style upper section harvested 2 days after controlled pollination and stained with analine blue. Pollen tubes (pt) extend for approximately one third to half the way down the style. Some pollen grains (pg) have not yet germinated. Bar = 100 μm.

bags with a wire coil to prevent damage to the emasculated buds. In 2000, this method was compared to the foil method where aluminium foil squares were cut approximately 4x4 cm and twisted around individual flowers after emasculation. Flowers isolated with foil squares did not require bagging. The aluminium foil squares were removed for pollination, and then replaced (Fig.1b). The foil method of isolation was used in 2001.

Emasculation and pollination

All buds to be pollinated were emasculated at the operculum lift stage, by cutting around the base of the anthers above the hypanthium with a scalpel, then removing all anthers and pollen from the bud, taking care to leave the disc, style and stigma undamaged (Fig. 1c) as described by Griffin *et al.* (1982). In 1999 it was found that flowers exposed to full sun to rarely set fruit

Table 1

Stigmatic receptivity of *Eucalyptus marginata* clones assessed from the numbers of pollen tubes in the style. 3–14 flowers were pollinated with processed pollen for each day followinganthesis ,

Days after anthesis	Range of numbers of pollen tubes (no. of flowers pollinated)			
	Clones (at Murdoch University, 1999)			
	5JN119 (November)	503JP16 (November)	11JN379 (January 2000)	
0	0 (10)	0 (10)	0 (10)	
1	0 (10)	0 (10)	0 (10)	
2	0 (10)	0 (10)	0 (10)	
3	1 – 5 (10)	1 – 5 (10)	1 – 5 (10)	
4	1 – 5 (10)	1 – 5 (10)	1 – 5 (10)	
5	0 (10)	5 – 10 (10)	1 – 5 (10)	
6	1 – 5 (10)	Not tested	1 – 5 (10)	
7	0 (10)	5 – 10 (10)	0 (10)	
9	1 – 5 (10)	1 – 5 (10)	1 – 5 (10)	
11	1 – 5 (10)	1 – 5 (10)	0 (10)	
	Clones (at Marrinup, 2000)			
	162A18 (September)	12JN72 (October)	133JN51 (November)	129J55 (November)
4		5 – 10 (4)	20 – 25 (8)	0 – 5 (6)
5	10 – 15 (7)			
6	25 – 30 (5)			
7	15 – 20 (7)			
	Clones (at Murdoch University, 2000)			
	503JP16 (September)	5JN119 (September)	503JP16 (October)	5JN119 (October)
3	0 (4)	0 – 5 (3)		
4	0 – 5 (3)		10 – 15 (5)	20 – 25 (5)
5	5 – 10 (12)		15 – 20 (13)	15 – 20 (14)
6	0 – 5 (3)			

when hand pollinated (data not shown) so that future work used only partly shaded flowers.

Statistical analysis

Chi-square tests, Wilcoxon matched pairs tests or log linear analyses tests were used at the 0.05 level of significance to test for significant differences (data was not normally distributed, discreet numbers were used in data collection, and sample sizes were small). All factors were fixed.

Results

Stigmatic receptivity

In the 1999 season, pollinating 5 – 7 days after anthesis gave the best results (Table 1) although some pollen tubes were observed in styles between days 3–11 after anthesis. In the 2000 season, pollinating 4–5 days after anthesis gave the most pollen tubes in styles, but best results varied over the flowering period being five days after anthesis in September (58% to 85% of flowers with pollen tubes), 4–5 days in October (58% to 81% of flowers with pollen tubes) and 4 days after anthesis in November (50% to 63% of flowers with pollen tubes). The interval between anthesis and maximum stigma receptivity apparently decreased as the weather warmed. In December 2001 when the wild trees at Wattleup were flowering, stigmatic receptivity followed four days after anthesis, where 86% to 100% of flowers were successfully pollinated (Table 2).

Pollen processing

The 'fresh' pollinating technique proved to be far more successful than using the traditional processed pollen.

Table 2

Percentage of styles containing pollen tubes in two wild *E. marginata* trees from Wattleup after control-pollination 4 days following anthesis in December 2001.

Tree	Number of flowers	% of styles containing pollen tubes
Wild tree 1	7	86
Wild tree 2	5	100

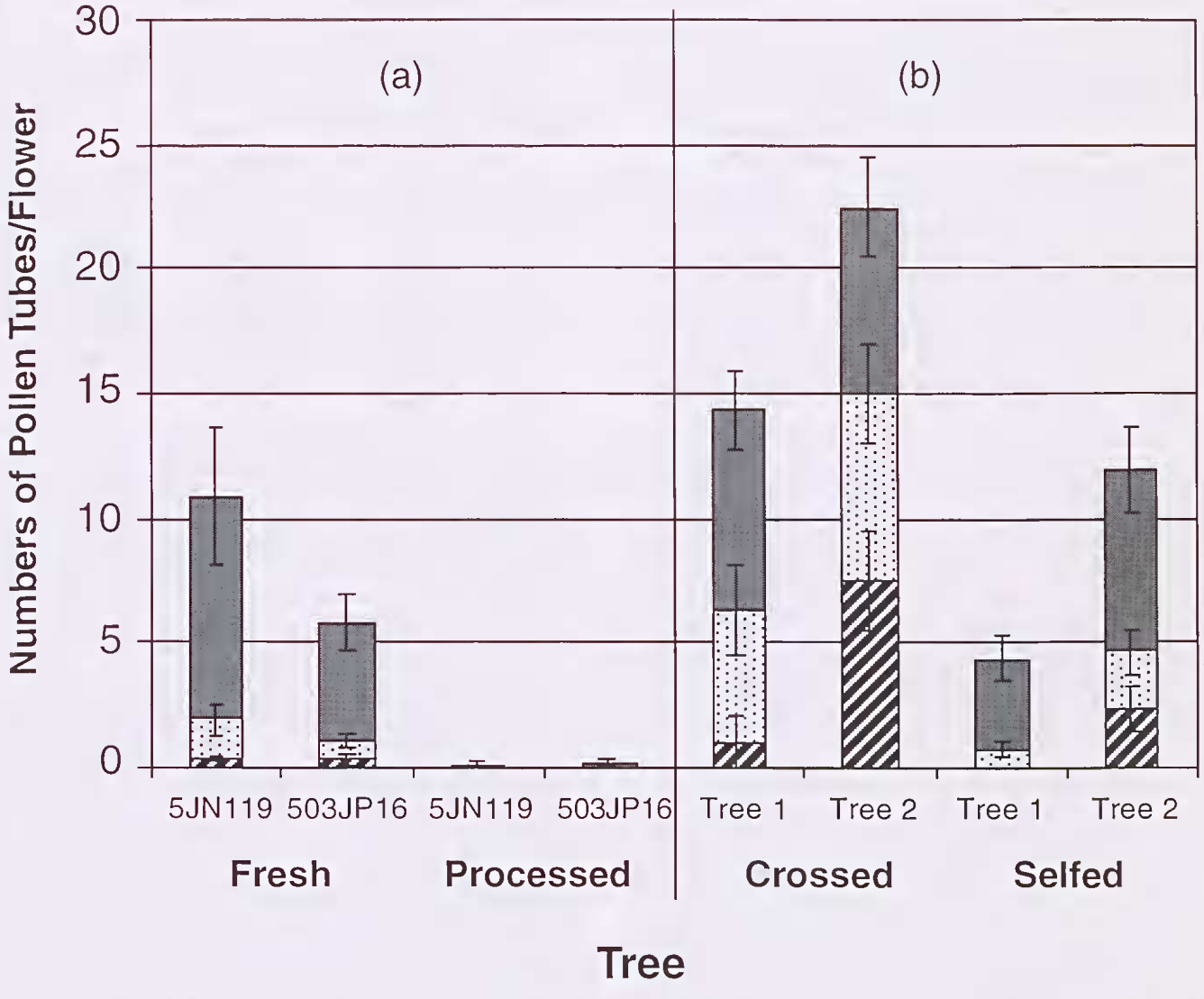

Figure 2. Comparison of mean numbers of pollen tubes in styles as a result of 'processed' and 'fresh' pollination techniques in controlled-pollination of jarrah (clone 5JN119 and 503JP16) flowers (2001 season, 28 flowers for each treatment), and of self- and cross-pollinated wild jarrah trees (2001 season, 3 flowers for each treatment). ▓ Represents tubes in the top third of the style; ⁚ represents tubes in the middle third of the style; and ▨ represents tubes in the bottom third of the style. Means are shown with standard error. Fresh pollen was used to pollinate buds 4 days after anthesis.

Numbers of pollen tubes in styles were greatly increased by using the 'fresh' technique, and some tubes reached the lower third of the style (Fig. 2). No tubes reached the lower third of the style using the 'processed' technique three days after pollination. A log linear analysis that considered technique, clone and position in the style found significant differences between the two techniques (Chi-square = 560.61, d.f. = 1, $p < 0.001$), clone (Chi-square = 41.43, d.f. = 1, $p < 0.001$) and position in the style (Chi-square = 501.32, d.f. = 2, $p < 0.001$), without any interaction between the three variables. There were also larger numbers of pollen grains adhering to the stigma (a mean of 39.5 pollen grains per style for clone 5JN119, and 17.2 pollen grains per style for clone 503JP16), pollinated using the 'fresh' technique. This is more than double the number seen using the 'processed' technique.

Pollination trials

The method of covering flowers with aluminium foil squares was faster to set up than the traditional method of isolating the branch with bags and wire coils. The foil squares were light, and good insulators from the hot sun. The use of two crispy-wrap bags with a wire coil for isolation tended to result in heat damage to the emasculated buds in the hot weather experienced in south-western Australia during the jarrah flowering season, and the bags were heavy on the small branches sometimes resulting in stem breakage.

From assessment of the number of pollen tubes in the styles there were no significant difference in pollination success between self- and cross-pollinated flowers in the 2000 season (0.05 level of significance, Table 3), but in the 2001 season, there were differences between selfed and crossed pollination results (Chi-square = 7.26, d.f. = 1, $p < 0.001$) that varied between genotypes (Chi-square = 4.22, d.f. = 1, $p < 0.001$), using the wild trees at Wattleup (Fig. 2). A log linear analysis with no interaction between the variables found significant differences between pollen tube length (Chi-square = 6.40, d.f. = 2, $p < 0.001$,), with the out-crossed pollen tubes being longer.

Table 3

Pollination success in self- and cross-pollinated flowers of jarrah clones (2000 season). The 'fresh' pollination technique was used to pollinate buds 4 – 6 days after anthesis.

	% of styles with pollen tubes (no. of flowers pollinated)	
Clone	Selfed	Crossed
503JP16 (Murdoch)	56.3 (12)	68.8 (18)
162A18 (Marrinup)	80.0 (5)	85.7 (6)
5JN119 (Murdoch)	80.0 (9)	66.6 (17)
Mean (& s.e.)	68.1 (7.32)	72.5 (13.51)

There were no significant differences in the numbers of pollen tubes between the two clones and two wild trees (Fig. 2). There was no significant difference between double pollinated flowers compared to single pollinations. No mature fruits resulted from the single or double pollinated flowers. No significant differences were found in pollination success between the early, middle and late season pollinations. The middle of the season produced most of the pollen tubes for both clones, with 5JN119 apparently remaining fertile for a longer period over the season.

Within three weeks of pollination wild tree 1 had dropped all the hand-pollinated fruits, but wild tree 2 held ten cross-pollinated fruits for at least eight weeks after pollination. No significant differences were noted between the number of self- and cross-pollinated fruits retained on trees six weeks after pollination (Table 4), but the wild trees held more cross-pollinated fruit than self-pollinated fruit at that point in time.

Very few fruits developed to maturity from all the controlled pollinations. While pollination was achieved in 2000, no hand-pollinated fruits survived longer than three months (Table 4), with the drop-off occurring at a consistent rate over the three months. Low numbers of mature fruits were obtained from the controlled pollinations of clones 5JN119 and 503JP16 in the season of 2001, with an average of 4.7% of pollinated flowers developing into mature fruits (Table 5).

Table 4

Numbers of fruits held by two wild trees and two clones six weeks after self- and cross-pollination (2001 season). 50 flowers were pollinated for each treatment on each tree. Fresh pollen was used to pollinate buds 4 days after anthesis. Wild trees were growing at Wattleup, and clones were growing at Murdoch University.

Tree	Self-pollinated flowers	Cross-pollinated flowers
Wild tree 1	0	0
Wild tree 2	3	13
Clone 5JN119	5	6
Clone 503JP16	6	5

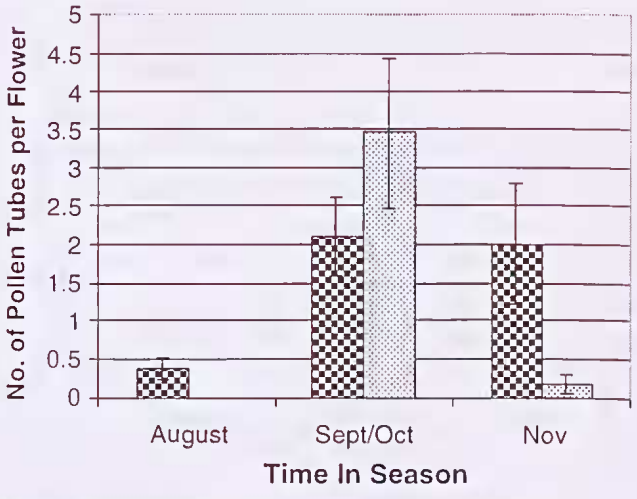

Figure 3. Pollination success at three different times over the flowering season of 2001 in two jarrah clones at Murdoch University. clone 5JN119, and clone 503JP16. There were 20 flowers in each treatment; means are shown with standard error. 'Processed' pollen was used to pollinate buds.

Discussion

This preliminary study suggests that successful control-pollination in *Eucalyptus marginata* may depend on a clearer determination of stigma receptivity and whether it varies from season to season. Observed stigma receptivity extended over 5 – 7 days with the peak of receptivity varying within and between the flowering seasons. Although no significant differences were observed in pollination success at different times of the season the results suggest that there may be greater pollination success in the middle of the season (Fig. 3).

The length of time taken for the pollen tube to reach the base of the style was also not established in this study. A possible reason for so few pollen tubes reaching the base of the style is that the styles were not left long enough on the tree after pollinating. Pound (2002) left the styles from pollinated *E. nitens* flowers on the trees for two weeks before harvesting and squashing. Pollinated styles were left for only two days in this work on *E. marginata*, but ten days probably would have been more appropriate. As Faegri & van der Pijl (1979) note, the

Table 5

Fruits resulting from control-pollinations in jarrah (2001 season). Open pollinated flowers were not emasculated.

Treatment	No. of flowers pollinated	Clone 5JN119 (% of flowers that produced mature capsules)	Clone 503JP16 (% of flowers that produced mature capsules)
Single pollinated	15	0	0
Double pollinated	15	0	2
Open pollinated	20	4	0
Early season	20	0	0
Middle season	25	0	2
End of season	10	0	0
Total	85	4 (4.7)	4 (4.7)

Table 6

Numbers of pollen tubes in *Eucalypt* styles. Data from *E. marginata* is sourced from work completed in 2000 (Table 1).

Eucalypt species	Mean number of pollen tubes observed in style
Eucalyptus marginata	13
E. globulus [1]	270
E. nitens [2]	48
E. woodwardii [3]	490
E. regnans [4]	160

1. Pound *et al.* (2002), 2. Pound *et al.* (2003), 3. Sedgley & Smith (1989), 4. Sedgley *et al.*, (1989).

self-incompatibility systems in plants may allow pollen tubes to grow for several days in the style.

There was no evidence for stylar self-incompatibility in jarrah as there was no significant difference observed in numbers of pollen tubes in styles between self- and cross-pollinated flowers (Table 4). This conforms to findings in other eucalypts (Ellis & Sedgley, 1992; Griffin *et al.*, 1987; and Sedgley *et al.* 1989), except for *E. woodwardii* (Sedgley & Smith, 1989), which showed a reduction in the number of selfed pollen tubes. Numbers of pollen tubes observed in styles were generally low when compared to other eucalypts (Table 6). However, numbers of pollen tubes in styles were increased with the introduction of the 'fresh' pollinating technique, and continued to increase as the work progressed.

The 'fresh' pollen-application technique was shown to be more effective for *Eucalyptus marginata* than the traditional 'processed' pollen method (Fig. 2). Fresh eucalypt pollen has not been widely used with cool temperate species such as *E. regnans* (Griffin *et al.*, 1982), *E. grandis* (Griffin *et al.*, 1982), and *E. nitens* (Moncur, 1995) because it will not germinate prior to desiccation. The hot dry flowering season in the jarrah forest may mean that the pollen is desiccated at anthesis, allowing it to germinate immediately. The 'fresh' technique increased control-pollination success compared to the 'processed' technique. One reason for this may be that sieving the pollen damages it, and another is that the storage overnight of the pollen, either at 4°C or at room temperature, may reduce its fertility (Wheeler 2004).

The low percentage of fruit maturation (4.7%) in the control-pollinations (Table 5) was surprising, and contrasts with other eucalypts where higher fertilisation rates have been achieved through controlled pollinations (Oddie & McComb, 1998; Pound *et al.*, 2002, 2003). The percentage of natural fruit set for clone 503JP16 was less than the percentage of mature fruits produced through controlled pollinations (1.5% for natural fruit set and 4.7% for control pollinated fruit, Wheeler, 2004). Fruit produced from the controlled pollinations was less than seen by Byrne and Stukely (*pers. comm.*) in 1996, who recorded 9.3% for crosses with clone 2J355 and 5.1% with clone 11JN550. They applied pollen with the 'processed' method and isolated flowers with bags and wire coils, methods that are considered here to be inferior to the 'fresh' pollen-application technique and foil isolation technique. They also double pollinated firstly on day three after anthesis and again on day six to day nine after anthesis, but in this study no advantages of double pollination could be demonstrated. If stigmatic receptivity extends over several days, as is suggested by the work presented here, double pollinating is unlikely to make a significant difference to the seed maturation percentage. Without any other factors being obvious, it is considered most likely that the differences seen within the experiments undertaken here, and between these pollinations and those undertaken by Byrne and Stukely represent female fertility differences between genotypes (Wheeler, 2004). Care must be taken in drawing conclusions when only one or two genotypes are used for research on a species that may contain thousands or even millions of genotypes in its natural distribution. The choice of clones or wild trees that have above average female fertility for use in controlled pollination studies would confirm whether the low fertilisation rates seen here were due to technique or to genotype. Clones also retained a lower proportion of flowers (5.5%) than wild trees (9.3%) from open pollinations to the mature capsules stage (Wheeler 2004), suggesting that there has been a change in the reproductive behaviour during the clonal process.

There are three possible reasons for the low seed maturation percentages seen in *Eucalyptus marginata* clones. The pollination rate (pollen tubes reaching the base of the style) may be low. This requires further investigation leaving the pollinated styles on the tree for longer before harvesting. The fertilisation percentage may be low, or the level of zygote abortion high. Most of the trees used for the controlled pollination work were clonal genotypes that may have different rates of pollination, fertilisation and zygote abortion to the wild trees.

Flowering in adjacent trees tends to be synchronous in *E. marginata*, as has been recorded in *E. regnans* (Griffin, 1980). However, the time of anthesis differed between the clones and the surrounding wild trees in the seasons of 2000 and 2001. The clones at Murdoch University (less than ten kilometres away) flowered approximately three months earlier than the wild trees. The variation in flowering periods between the clones and wild trees was difficult to explain. Clones were derived from wild trees (5JN119 – Harvey, 503JP16 – Nannup, 11JN379 – Yanchep, 162A18 – Collie, 12JN72 – Kirup, 133JN51 – Jarrahdale, 129J55 – Jarrahdale), which with the exception of Yanchep are further south than Perth, yet they flowered much earlier than the neighbouring wild trees in the years 2000 and 2001, so that there was little, if any, overlap in flowering between them. In the years 2000 and 2001, the Murdoch clones had almost completed flowering in the second half of November, but the wild trees nearby and at Wattleup (approximately 10 kilometres away) did not begin flowering until December. Older jarrah trees tended to flower later than younger trees, as seen in *E. regnans* (Ashton 1975), but young wild trees were used at Wattleup, so age should not be a factor in the difference in flowering times between the clones and the wild trees. The soils at Murdoch University are sandy, whereas the wild trees that the clones are derived from all grow on serpentine soils, except for Yanchep. The clones at Murdoch University are not irrigated, so moisture should be approximately equal to the wild trees. There may be

differences in micro-organisms between the clones and wild trees. The differences in climate when the trees were flowering made it difficult to draw conclusions regarding the timing of stigmatic receptivity in the clones and the wild trees.

Future work is required on events at fertilisation and during early zygote development to explain the low levels of seed set following hand-pollination. More detailed stigma examination to determine characteristics and duration of receptivity, as has been undertaken with other species (Ellis & Sedgley 1992; Sedgley & Smith 1989) could also be of value..

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